The Protective Effects of some Narcotic Substances Against X-irradiation on Aquatic Animals (III) Effects of Urethane and Changes in Concentration of Chlorobuthanol Solution Against X-irradiation in Larvae of Rhacophorus arboreus

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両生類に対する麻酔剤の放射線保護効果 (III) Urethane 及び種々濃度の chlorobuthanol の Rhacophorus 幼生に対する放射線保護効果について

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生体の放射線感受性は生体，細胞の酸素圧によつて変化しうるであろうことはよく知られており，酸素在存下の照射よりも無酸素状態で照射されることによつて放射線感受性が低下することはよく知られた事実である．また，いくつかの麻酔剤や鎮痛剤も放射線保護剤として知られている．本研究に用いた，urethane および chlorobuthanol は水生動物の麻酔剤として知られている．Rhacophorus 幼生に1～5％の chlorobuthanol と1％の urethane を照射前，照射中に処理して，体重の変化，生残曲線を指標として，保護剤の効果をしらべた．この結果は urethane において，生残率の高度な曲線，体重における，非薬剤処理との比較での体重増加がみとめられた．一方，chlorobuthanol では，1％溶液では放射線保護效果がえられなかつたに反し，2および5％においてはあきらかな保護效果がえられた．濃度変化による效果の差異がしめされたが，2％以上で保護效果のあることをみとめた．これらの效果の機作について検討を加えた．

INTRODUCTION

It is known that radiosensitivity of living matter depends on the oxygen tention in the cells or in the organisms.

In the numerous studies, radiation injury was confirmed to be greater under the presence of oxygen than in anoxic states during irradiation. However, little is known about the mechanism of oxygen effect and it must be considered a key to the solution of the problems of radiation effects.

Some narcotic substances and analgetics have been known as weak protectors (Bacq and Alexander ('61[1])., Pomeranlseva ('58[2])., Cole et al ('52[3])., Paterson et al ('51[4])., and others).

According to Tanaka[5], narcosis with ethyl alcohol and chlorobuthanol of 5% solution in aquatic animals increased the survival rate and rate of regeneration of the tail.

Previous studies by Cole et al('61[6]) have shown that the radioprotective effect of urethane was not seen when the drug was administrated 30 minutes before irradiation, nor when the mice were irradiated 7 days after the last urethane injection. But, when the urethane was administered 1 or 2 days prior to whole body X-irradiation, an appreciable increase in survival rate was observed.

Urethane has a narcotic action on aquatic animals as well as does chlorobuthanol.

The author studied the effects of 1%, 2%, and 5% chlorobuthanol and 1% urethane on the survival response and on body weight in larvae of Rhacophorus arboreus.

MATERIALS AND METHODS

Rhacophorus arboreus were obtained near the university in Kyoto City in an egg block.

The animal used in the urethane treatment and chlorobuthanol treatment experiments measured about 26–27 mm and 22–23 mm, and were developed from the same egg block respectively. Each group consisted of 25 animals and totalled 300, in all.

Death were recorded twice a day for a total of 24 days (chlorobuthanol treatment group) and 27 days (urethane treatment groups) after X-irradiation. Measurements of body weight were made on 10 randomly selected animals from each experimental group immediately after irradiation and at 5, 10, 15 20 and 24 days thereafter (in chlorobuthanol groups) and 6, 10, 14, 18 and 22 days thereafter (in urethane groups).

The tadpoles were irradiated in a plastic-covered box. Before and during irradiation, the animals were treated in the drug solutions (chlorobuthanol and urethane) and water (only irradiated control).

The radiation was delivered at a target-surface distance of 10 cm, with factors of 80 kvp. 4 ma, and a dose-rate of 300 R per minute.

The experimental methods were as follows.

A. Experiment of 1% urethane treatment

1) 5 minutes prior to and during (6 minute) X-irradiation soaking in 1% (urethane (1800 R)

2) X-irrdiated control in water only (1800 R)

3) 5 minutes prior to and during (3 minute) X-irradiation soaking in 1% urethane (900 R)

4) Treatment of 1% urethane only without irradiation

B. Experiment of 1%, 2% and 5% chlorobuthanol treatment

1) X-irradiated control only (1200 R)

2) 5 minutes prior to and during (4 minute) X-irradiation soaking in 1% chlorobuthanol solution (1200 R)

3) 5 minutes prior to and during (4 minute) X-irradiation soaking in 2% chlorobuthanol solution (1200 R)

4) 5 minutes prior to and during (4 minute) X-irradiation soaking in 5% chlorobuthanol solution (1200 R)

5) 5% chlorobuthanol solution soaking for 10 minutes only

6) Non-irradiated, non-drug treated control

7) 0.5% (45 minutes)—1% (30 minutes)—2% (5 minutes)—5% (10 minutes) soaking in chlorobuthanol before X-irradiation and X-irradiated in water (1200 R)

RESULTS

A-1. Survival response of 1% urethane treatment.

Data obtained from these methods are shown in figure 1. These resultsindicate a significantly greater

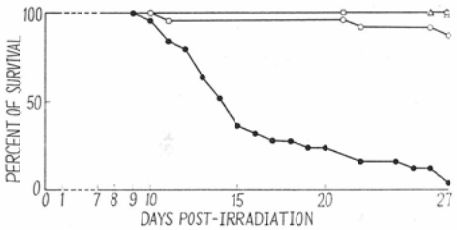

Fig. 1. Survival response in 1% urethane treatment.

○——○ 5 minutes prior to and during (6 minutes) X-irradiation soaked in 1% urethane (1800R)

●——● X-irradiated only ctontrol in water (1800R)

△——△ 5 minutes prior to and during (3 minutes) X-irradiation soaked in 1% urethane (900R)

□——□ Non-irradiated, treatment of 1% urethane only

degree of protection in the 1% urethane treated group than in the irradiation only control group. As shown in figure 1, the median survival time (50% lethal time) of the irradiated control group was 14 days after iradiation, whereas, the survival rate (27 days after irradiation) of 1% urethane soaked group was approximately 90%. Furthermore, the survival rates of two non-irradiated groups (with and without urethane treatment) were 100%.

Any possible developmental injury caused by 1% urethane was not seen at all in this experiment.

A-2. The response of body weight to 1% urethane treatment after X-irradiation.

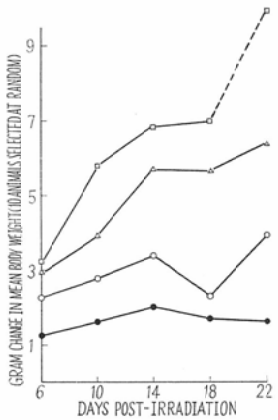

Fig. 2. The response of body weight in 1% urethane treatment.

····· metamorphosis occurred (referenced record)

○——○ 1% urethane treatment+1800R

●——● Irradiated only control (1800R)

△——△ 1% urethane treatment+900R

□——□ Non-irradiated treatment of 1% urethane only.

As shown in figure 2, the measurements of average body weight began at 6 days and ended at 22 days after irradiation. Being collected from the same egg block, the average body weight was the same before X-irradiation in all groups. As seen from the figure, depression of body weight increase was already shown 6 days after irradiation in the X-irradiated, non-treated group. The most prominent increase of body weight was seen in the non-irradiated, treated group, followed by the irradiated, urethane soaked groups.

Thus, the protective effects of urethane were shown in both survival response and mean body weight.

B-1. The survival response with varying concentration of chlorobuthanol.

The survival curves of Rhacophorus tadpole irradiated in various concentrations of chlorobuthanol, solution (0,1,2, and 5) are indicated in figure 3. However, these in the over concentration of chlorobuthanol, the survival. rate of the last day (24th day) was 84% (2% conc.) and 76% (5% conc.) each others. Further, no deaths occurred in the non drug treated and drug treated non irradiated groups.

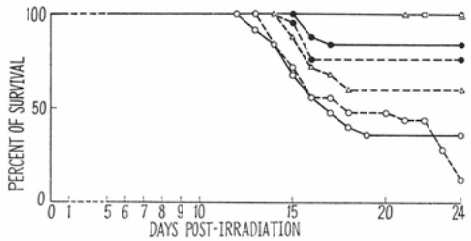

Fig. 3. The response of survival by change in chlorobuthanol concentration

- O——O Irradiated only (1200R)
- O·····O 5minutes prior to and during (4minute) X-irradiation soaked in 1% chlorobuthanol (1200R)
- ●——● 5minutes prior to and during (4 minute) X-irradiation soaked in 2% chlorobuthanol (1200R)
- ●·····● 5minutes prior to and during (4minute) X-irradiation soaked in 5% chlorobuthanol (1200R)
- □——□ 5% chlorobuthanol solution soaked for 10 minutes only
- △——△ Non-irradiated, non-drug treatment control
- △·····△ Soaked in various concentrations of chlorobuthanol before X-irradiation and X-irradiated in water

As is shown in figure 3, the group which was irradiated in water after aneasthesis was recorded in median value between the irradiated only group and the 2,5% chlorobuthanol solution soaked groups.

Thus, since the protective effect of the chlorobuthanol soluion depends on the degree of concentration, from the data in 2% and 5% treated, by over the certain concentration were demonstrated protective effect against X-irradiation.

B-2. Response of mean body weight.

Figure 4 indicates the effects of X-irradiation and drug treatment on mean body weight 10 ramdomly selected animals. Body weight increased a little more in the chlorobuthanol soaked group (2 and 5% concentration) than in the irradiated-only group and 1% drug treated group. On the contrary, the two non irradiated groups demonstrated normal growth.

DISCUSSION

Previous studies by the author have demonstrated the radioprotective effect of chlorobuthanol and

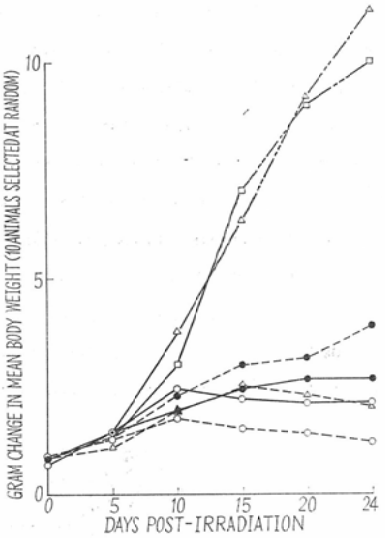

Fig. 4. Response of mean body weight

..... metamorphosis occurred (referenced record)
○——○ Irradiated only control (1200R)
○·····○ 1% chlorobuthanol+1200R
●——● 2% chlorobuthanol+1200R
●·····● 5% chlorobnthanol+1200R
□——□ 5% chlorobuthanol soaked only
△——△ Non-irradiated, non-drug treated control
△·····△ Various chlorobuhtanol+water (1200R)

ethyl alcohol solution on aqutic animals.

In these experiments, the survival response to 1% urethane and 2 and 5% chlorobuthanol solutions demonstrated a radioprotective effect by comparison with irradiated-only groups.

According to Vacek et al ('64[7]),the tension of oxygen in various tissues of mice was studied during irradiation at a dose-rate of 200 R per minute and upon administration of narcotics (thiopental). At the same time, the consumption of oxygen was followed up after both procedures. Results showed that during irradiation there was a decrease of oxygen tention in the muscle and spleen of the narcotized mice and the oxygen consumption in mice irradiated at a rate 200 R per minute also decreased.

Furthermore, narcosis and its effects on irradiation in mice was studied by Pomerantseva('58[8]). According to his experiments, narcosis with nembutal barbamyl and ether of mice X-irradiated with 500 R increased survival rate, prolonged survival and resulted in a weight gain. He maintains that the protective effect of the narcotics was due to the inhibition of the respiratory center which resulted in hypoxemia.

In addition to these finding, it is known that various agents have radioprotective effect on mammals, for example; ethyl alcohol (Cole et al ('52[3]), Paterson and Matthews ('51[4])), Urethane (Cole et al ('61[6]) etc), 2, 4-dinitrophenol (Praslicka et al ('62[9])) and others.

On the other hand, it is understood that the effects of γ-irradiation on the chemical composition of narcotic substances (halothane, ether, divinyl ether, etc) produced no qualitative or quantitative changes (Lawrence et al ('65)[10]) and no difference was found between the dissolved oxygen value of the tested

water and that of 20% chlorobuthanol solution (Tanaka[11]).

In this experiment, the highest degree of radioprotection, in terms of 24 days and 27 days survival, and a little radioprotection in the body weight, was observed in the amphibian tadpoles which were soaked in 1% urethane and 2 and 5% chlorobuthanol solutions. Since oxygen consumption in the individual tissue of the organism is related to anaesthetic action, it can be assumed that the changes in whole body oxygen consumption will also show themselves by a deflection of oxygen-consumption in various tissues, which should be acompanied by changes in oxygen-tention of the tissues.

As stated in previous paper Tanaka[5],[11], from the limited data of these experiments the oxygen effect cannot be assumed to be the sole factor in radioprotection.

However, from the results of these experiments, the protection of aqatic animals to irradiation, at least, is clearly demonstrated. Therefore, as a result of reduction in the respiratory metabolism by anasthetic action, the major factor of the radioprotective effects in these narcotic substances may be connected to the low oxygen tention in the organism and furthermore may be related to the chemical properties of these narcotics.

SUMMARY

1) The highest degree of radioprotection, in term 27 days survival, and a little radioprotection in the body weight growth were observed in Rhacophoru tadpoles which were soaked in 1% urethane 5 minutes prior to and during (6 minute) X-irradiation.

2) Radioprotection in survival was observed in Rhacophorus tadpoles which were soaked in 2 and 5% chlorobuthanol solutions, but was not observed with 1% chlorobuthanol. The radioprotective effect on the developmental growth of body weight with the above mentioned narcotic substances (2 and 5% concentration) was observed to be slight.

It is concluded that the mechanism of the radioprotection on the various narcotic substances used in the present experiments is probably caused by the changes of the oxygen content of the cells and considerably reduced oxygen consumption. However, other biological factors, for example the nervus system etc, must not be overlooked and moreover, the chemical properties of these narcotics may be related causes.

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